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We have developed a unique magnetic resonance imaging multi voxel pulse sequence unaffected by intravenous contrast producing spectroscopic images of key metabolites (choline) found in breast cancer, and validated our work in 22 breast lesions with in vitro spectra and pathology. Choline peaks are present in 57% of breast cancers but contrary to other research data, some invasive ductal cancers do not contain choline as a detectable metabolite, particularly in lobular cancer that has dispersed cells. Furthermore, benign fibrocystic change/fibrosis, hamartomas and patients with silicone injections may have detectable choline from increased metabolic activity not due to malignancy. Thus, technical developments to date have significantly contributed towards the goal of making MR spectroscopic imaging a clinically useful procedure that could be implemented at the time of a contrast-enhanced MRI scan, but would be helpful only in choline positive cases to prompt biopsy.

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INTRODUCTION

Magnetic Resonance Spectroscopy (MRS) has emerged as a powerful, non-invasive method for studying tumor biochemistry and has the potential to improve the specificity of contrast-enhanced breast Magnetic Resonance Imaging (MRI). Historically, the phosphorous (^{31}P) nucleus has been most commonly observed in experimental MRS studies of tumors to demonstrate metabolite concentration differences between tumors and normal tissues[1]. Two of these ^{31}P MRS observable compounds, PC and GPC, contain N-trimethyl groups in the choline moiety that are visible in a ^{1}H MR spectrum. The N-trimethyl group contains 9 equivalent protons in choline[2], producing a single resonance at 3.2 ppm arising from all choline-containing compounds. Several in vivo and in vitro studies have used elevation of this peak to discriminate between normal and neoplastic tissue[2-9]. It is postulated that the concentration of unbound choline-containing compounds increases with cell membrane synthesis and/or number of cells leading to an elevated choline peak at 3.2 ppm. High field strength proton MRS studies of malignant and normal breast tissue extracts suggest that this signal is derived largely from phosphocholine[7-10]. The purpose of our study was to evaluate an in vivo MR spectroscopy method that incorporates automated shimming and enhanced in vivo water and lipid suppression for differentiating benign and malignant breast lesions by detecting an elevated choline peak in malignancies. The gold standard to confirm the presence of choline was an in vitro MR spectroscopy of breast cell samples. Finally, the MRS data were to be correlated to biopsy results.

The specific aims of this project were as follows:

Specific Aim 1: To develop and optimize a new in vivo multi-voxel proton MRS technique to measure choline peaks in breast cancer.

Specific Aim 2: To demonstrate that in vivo MRS can distinguish cancer from benign breast disease. We hypothesized that in vivo MRS choline measurements would be elevated in breast cancer compared to benign disease. The optimized MRS sequences developed in Year 1 were used in patients with breast cancer and benign disease in Years 2 and 3. The in vivo MRS data was compared to both final histology/cytology and in vitro MRS scans of breast cell aspirates.

BODY

Our work was divided into a 3-year study, which was separated into two phases: (Phase 1) technical development and optimization in Year 1 corresponding to Specific Aim 1 and (Phase 2) clinical testing in the Years 2 and 3 corresponding to Specific Aim 2 . This final report covers the results of this three year project.

Year 1

During year 1 we planned for MRS technical development and optimization to:

1. Develop and optimize high resolution in vitro MRS in phantoms
2. Develop and optimize the in vivo MRS pulse sequences in phantoms

In year 1 we were to recruit and study normal volunteers and 5 breast cancer patients to further test, develop and validate the optimized in vivo MRS pulse sequences and the high resolution in vitro MRS of tumor cells obtained by fine-needle aspiration on cancer patients.

The first year involved MRS pulse sequence development and optimization for detecting choline while limiting water and lipid signals, and minimizing motion-induced artifacts which can interfere with choline peak detection. Pulse sequence development involved testing and optimization in phantoms and volunteers. We developed a method to analyze breast cells using a high resolution in vitro MRS study in a Stanford University chemistry laboratory to validate the in vivo MRS data, optimizing our protocol with standards. We hired a nurse coordinator to recruit patients and developed a clinical protocol for MRS scans and patient transportation to the hospital for breast tumor fine-needle aspirations of the breast tumor under direct ultrasound guidance. Given our rapid pulse sequence development, we began early patient recruitment in Year 1 for clinical test scans that were to be done in Years 2 and 3. We recruited patients with suspicious breast lesions and performed a contrast-enhanced diagnostic MRI to localize the lesion. We then used the optimized MRS pulse sequence on the breast mass in the MRI scanner, performed a fine-needle aspiration of the tumor to obtain breast cells in the hospital, used the optimized in vitro MRS study on the cells, then correlated the MRS studies to the cytology/pathology and in Year 1 we reported preliminary data on 5 patients. The details of our most significant accomplishments in Year 1 are as follows:

In year 1 our personnel infrastructure changed upon the departure of Josh Star-Lack Ph.D. from Stanford University. Sandeep Hunjan, Ph.D., replaced Dr. Star-Lack in October 1999 to develop and optimize in vivo MR spectroscopy methodology for choline imaging in breast cancer, and to optimize the parameters for the high-resolution in vitro MRS scans of phantoms and breast biopsy samples. A second important addition was the hiring of Leslie Jerome-Roche, R.N as nurse coordinator to optimize contacts with referring physicians and to help with patient recruitment. Detailed aspects of this work are described below.

In vitro MR spectroscopy development, optimization and testing: We developed a choline, creatine, and NAA phantom for in vitro high resolution MRS to optimize parameters for acquiring high resolution spectra in the wet laboratory. MR spectroscopic experiments were performed at 400 MHz or 9.4 T equipped with a Varian console and a 5-mm probe. Spectra were obtained with good signal to noise ratios (SNR) in 4 acquisitions with this phantom.

To obtain breast cell samples for the high resolution MRS in vitro studies in patients, informed consent was obtained to perform needle biopsies after the MRI/MRS scans. Patients were accompanied to the hospital immediately after the MRI / MRS scans. In the hospital, the breast lesions were biopsied with a fine needle aspiration (FNA) technique under direct ultrasound guidance after injection of local anesthesia and using sterile technique. Each biopsy obtained breast cell samples using sterile 21 gauge needles, and usually 4 samples were obtained with four separate needles. Alternatively, within 6 weeks of the MRI / MRS scan, cells were obtained from the mass in the operating room by fine-needle aspiration under direct palpation of the operated specimen, and again, usually 4 samples were obtained with four separate needles. FNA samples were immediately placed into polypropylene vials that contained chilled phosphate buffered saline (PBS) in D2O. All specimens were then immediately immersed into liquid nitrogen and stored at –78 degrees C for up to 6 weeks until MR spectroscopic analysis could be performed. One sample was stored for 7 weeks. Before in vitro 1H MRS, each FNA sample was thawed and transferred to a 5-mm MR Spectroscopic tube under a vented hood in a wet laboratory. The volume was adjusted to 400 ul with PBS in D20. The samples were spun at 20-25 Hz, and the temperature was maintained at 37 degrees

C. Residual water signal was suppressed with selective gated irradiation by using the decoupler placed on the water resonance. Proton spectra were acquired with the following parameters: sw = 5000 Hz, pw = 29 us (90 pulse), 256 free induction decays, acquisition time = 1.14 s and relaxation delay = 2 s (TR = 3.14 s). MR spectra of FNA specimens were processed using "NUTS" shareware running on a PC using MS Windows. A 3 Hz line broadening was applied to the data before Fourier Transformation followed by phase and baseline.

In vivo MRS pulse sequence development and optimization: As part of our efforts to improve our ability to collect proton spectra from the breast in patients for the in vivo portion of the experiment, new pulse sequences designed to more robustly separate choline and lipid were developed and evaluated in phantoms. MRS pulse sequence development also included automated shimming. We redesigned the RF pulses for improved lipid and water suppression using dual BASING techniques. During pulse sequence development and testing in volunteers and in patients, we encountered considerable artifacts due to B1 and B0 inhomogeneities near the chest wall and heart, and in large patients where the breast touched the RF coil, resulting in "hot spots". To correct these "hot spots", we added highly selective saturation bands that were used to suppress these tissues giving rise to the artifacts. The resulting diagnostic MRI sequence and optimized MRS sequence resulted in a 90 minute scan time, requiring an overall 2-hour scan time slot for scan set-up, scan completion and archiving.

We developed and implemented a program and protocol to identify and recruit appropriate breast cancer patients from the Surgery, Oncology and Radiation Oncology departments using a nurse-coordinator, Leslie Jerome-Roche, RN. We used the pre-tested, optimized diagnostic MRI and MRS scans on subjects after informed consent (approved by the Stanford University IRB). All MRI and MRS examinations used a four channel phased-array breast RF coil (MRI Devices) and all scans were obtained on a conventional 1.5 T GE Signa scanner. Patients were initally scanned with an MRI pulse sequence to locate the lesion employing a high resolution three dimensional spectral spatial magnetization transfer pulse sequence (3DSSMT) [11-13] which provides high resolution morphologic data immediately following a rapid 3D spiral sequence which produces 3.5 minutes of kinetic data of breast lesion enhancement 3D spiral scan parameters included 20-interleave spirals, 20 cm FOV, TR/TE 38/11.9 ms, 500 flip angle, 4.5 to 6 mm thick slices, 188x188 matrix, 20 slices volume, scan time of 10.86s. 3DSSMT parameters included TR/TE 33-40/7 ms, 512x192 matrix, 60 slices, scan time of 1.0-2.0 min., using centric phase encoding, water selective excitation and on-resonance magnetization transfer pulse [14]. In cases in which intravenous contrast could be used, we used 1 mmol/kg intravenous gadolinium given as a bolus during the initial spiral sequences and flushed immediately with 20 cc of sterile saline.

To obtain the MRS spectra we used spiral k-space trajectories (hereafter called spiral CSI [15]) with which we obtain both volumetric metabolite data and a rapid water reference acquisition used to automatically phase and quantify the spectroscopic data and extended spiral CSI to allow the acquisition of spatially resolved 2D NMR spectra. Details of a J-resolved spiral spectroscopic imaging sequence is given in [16]. The data processing for these spectroscopic studies is based on our previous work on signal estimation and water referencing using prior knowledge [17, 18].

We initially used a single-voxel MRS acquisition technique known as point resolved spectroscopy (PRESS [19]) on our first patients. The PRESS technique consists of three slice-selective excitation pulses along orthogonal axes, using presaturation for water suppression[20]. Our improved PRESS sequence using spectral-spatial excitation pulses achieves a significantly improved uniformity of metabolite intensities within the PRESS box along with decreased contamination by signals outside

the volume of interest [21]. In addition, we achieved improved water and lipid suppression using an independently developed method known as band selective inversion with gradient dephasing (BASING[22]]. We acquired in vivo spectra of choline resonances by developing a two-shot J-difference single-voxel editing technique incorporating the BASING pulse sequence into PRESS localization. The technique requires two acquisitions in which the lactate methine quartet is inverted by the BASING pulses in the second acquisition but not the first. Sum spectra then yield the uncoupled singlets, including creatine, choline, and lipids, while difference spectra yield the lactate methyl doublet (1.3 ppm). Motion artifacts are minimized by tracking the frequency and phase of the residual lipid peaks. This artifact reduction technique results in lipid suppression factors of over 1000.

As part of our overall MRS program, 9 patients with suspicious breast masses or mammographic findings were studied with high-resolution breast MRI examinations (7 contrast-enhanced, 2 non-contrast) and MRS during the first year. As part of the Stanford MRS program, single-voxel scans were obtained on the first patients as part of the program to develop multi-voxel sequences used for this study; of 5 patients undergoing the multi-voxel MRS sequences to date, two cases were technically suboptimal and were excluded from the overall spectroscopy data. The suboptimal cases and the single-voxel cases led to development of the highly selective saturation bands needed to decrease artifacts from the heart/chest wall and in large breasts, and also led to further improvements in the multi-voxel sequences. Furthermore, use of the initial single-voxel cases validated use of the contrast-enhanced breast MR images to use as a guide for correlating the location of the tumor within the breast to the tumors biopsied under direct ultrasound visualization for fine-needle sampling. Lastly, use of the single-voxel and multi-voxel sequences in the same patient helped to develop and confirm presence of choline in the same location within the breast (Appendix 1).

Using correction factors obtained from the pre-scan transmitter gain and by optimally combining data from each of the coils, we were able to obtain quantitative measurements of choline levels within the breast lesions during the MRI/MRS scan. Subsequent to each MRI/MRS study, all lesions underwent tissue aspiration from suspicious breast masss using a 21 gauge needle (4 needle passes in 7 patients, 6 needle passes in 2 operated breast specimens). Direct ultrasound guidance was used to direct the needle to obtain cell samples in 7 patients immediately after the scan. In 2 patients, aspirates were taken directly from breast tissue in the operating room. In these 2 cases the needle was directed into the mass for cell removal by physician palpation immediately upon tissue removal during surgery.

Our technical accomplishments in the first year also included continued optimization of the MRS techniques for measuring in vivo choline levels in the breast. This was accomplished at three levels: pulse sequence development, optimization of RF coil configurations, and improved data reconstruction and quantification algorithms. In particular, a PRESS sequence was modified with the addition of dual-band BASING pulses for added water and lipid suppression. Sharper delineation of the excited volume of tissue was achieved with the addition of up to 6 graphically prescribed highly selective spatial saturation bands. Our phantom and patient data showed excellent suppression of water and lipid resonances while passing the choline and/or creatine resonances with no detectable attenuation. Several Rf coils were investigated include volume, surface, and phased array coils. The best results were obtained with a 4-coil phased array breast coil configured to only receive signal from the right or left side respectively depending on the location of the selected lesion.

Finally, in vivo data was processed using custom software to both detect and correct motion-induced phase variations followed by peak integration.

In summary, in Year 1 we accomplished much of the pulse sequence development and infrastructure building for scanning patients and performing the fine-needle biopsies. In our annual report in Year 1 we reported on the results on 5 patients.

Years 2 and 3

In Years 2 and 3 our tasks were to:

1) Perform MRI and MRS scans on 5 patients with breast cancer and 5 patients with benign lesions in each year of Years 2 and 3
2) Correlate the imaging studies, cell aspirates, MRS and pathology
3) Perform data entry and data analysis

During Years 2 and 3 we processed MRS data on scanned patients from Year 1, developed volumetric spectroscopic imaging techniques to acquire short-echo-time in vivo proton spectra thereby increasing the signal to noise ratio for choline. In our preliminary data we noted that the dual-BASING PRESS pulse sequence obtained excellent water suppression along with adequate suppression of the 0.9 and 1.3 ppm lipid peaks. However, additional lipid resonances, particularly between 2.8-2.3 ppm were not fully suppressed, and, in some cases, obscured the choline peak of interest. Pulse sequence improvements in Year 3 moved the echo time out from TE = 144 ms to TE = 288 ms, aimed at improved lipid suppression.

Due to rising costs at the Lucas Center, the actual cost of scanning each patient limited the number of scans we could perform, and we were ultimately limited to scanning 23 patient breasts due to fund limitations. We scanned a total of 22 patients with 23 breasts and performed fine-needle aspiration on all cases.

We scanned breast cancers both treated and untreated with chemotherapy, and with and without intravenous contrast to determine if cancer treatment or if the presence of contrast affected in vivo MRS data collection. It has been postulated that Gadolinium-based (Gd) contrast agents may broaden the choline peak resulting in loss of choline signal and inaccurate quantitation of metabolites[23-24]. We acquired our MRS data approximately 25 minutes after initial Gd infusion due to time needed to acquire the diagnostic MRI scans (3DSSMT and spiral dynamic pulse sequences). Using our MRI and MRS techniques, we found that intravenous gadolinium infusion did not significantly affect MRS choline peak detection.

Regarding the in vitro MRS scans and technique, in Years 2 and 3 we focused on increasing the yield of viable breast cell samples on fine-needle aspiration because of our unexpected finding of no choline peak on the in vitro MRS scan in a patient with a large treated breast cancer that showed an elevated choline peak on the in vivo MRS scan. We postulated that the absence of choline peaks on the in vitro MRS scan was due to breast cell samples from a necrotic part of the tumor (since a non-viable treated portion of the tumor is indistinguishable from living cancer on the ultrasound). We increased the sample aspirate by sampling both the center and the periphery of the tumors to increase the probability of obtaining live tumor cells, added a hand-held aspiration device to increase suction during the needle biopsy thereby increasing cell yield, and increasing the total number of excursions through the tumor (15-25 excursions per needle) while not increasing the total number of separate needle sticks into the patient (four needles in total). We obtained the pathology and cytology data on scanned patients and correlated these to the in vivo and in vitro spectroscopy.

Results

Our prior annual and midterm reports had included our preliminary data in which MRS scans under development were obtained in 4 breast cancer patients. These 4 patients were positive on the in *vivo* MRS scan but they did not undergo fine-needle aspiration for the in vitro confirmatory MRS studies. Similarly, we scanned normal volunteers on preliminary MRS scans, with negative results, and these women also did not undergo fine-needle aspiration as per our Statement of Work. To review the original Statement of Work which was to report the findings on both in vivo and in vitro MRS this final report includes only patients who were scanned with the MRS pulse sequences and in whom FNA data was also obtained. We have incorporated new information on previously scanned patients, including measurements of the tumor sizes when available on the pathology reports, and revision of the histology as it was reviewed by the pathologists, which changed one of the previously reported cases of possible invasive ductal/lobular cancer to invasive ductal carcinoma only.

22 patients underwent both in vivo MRS and fine-needle aspirations for in vitro MRS. Of these 22 patients, 17 patients had cancer (1 with cancer and a benign phyllodes tumor) and 5 with benign tumors. MRS data was irretrievable in 1 cancer patient due to a computer malfunction. In 4 patients (2 cancer, 2 benign) choline peaks were obscured due to extreme patient motion during the scan, poor lipid suppression, or problems with the sequence during scanning.

Our data are shown in the Appendices in Table 1. In vitro and in vivo results are indicated as a positive or negative for an increase in the N-trimethyl resonance of choline at 3.2 ppm (arising predominantly from the choline of phospholipids). Of 21 patients with 22 scans, 5 patients had benign lesions (post-biopsy scar, fibroadenoma, fibrocystic change/stromal fibrosis, hamartoma and silicone granuloma); 1 patient had invasive ductal cancer and a benign phyllodes tumor, and the remaining 15 patients had invasive ductal cancer (11), inflammatory cancer (2), both invasive ductal and lobular cancer (1), and invasive ductal carcinoma with mucinous features (1). Of the 16 cancers, 14 had technically satisfactory in vivo MRS scans and 8 (57%, 8/14) of these technically satisfactory in vivo scans were positive for choline. Most (6/8, 75%) of positive in vivo MRS scans were also positive on in vitro MRS confirmation except for 2 patients who had negative in vitro MRS scans (1 patient with a very bloody FNA sample, and 1 patient who had invasive cancer with mucinous features).

6 of the 14 (43%) technically satisfactory scans in cancer patients were negative on in vivo MRS scans. Of these 6 cancer patients, 5 (83%, 5/6) were also negative on in vitro MRS confirmation. The 1 patient with a negative in vivo MRS scan and a positive in vitro MRS confirmation had a tumor with both invasive ductal and lobular histologies. The other 5 negative in vivo MRS cases had invasive ductal cancer.

There were 4 technically satisfactory in vivo MRS scans of the 6 patients with benign disease and none had choline on the in vivo MRS scans. 3 of 4 (75%) had no choline peak on the confirmatory in vitro MRS scans. Positive in vitro MRS scans were seen in three patients with benign disease with negative (1) or technically unsatisfactory (2) in vivo MRS scans. The positive in vivo MRS scans were seen in fibrocystic change and dense fibrosis, a hamartoma and in a patient with silicone granulomas from direct silicone breast injections for breast augmentation.

Discussion

Our first four preliminary scans had shown choline peaks in all 4 of our test cases in large breast

cancers, and we expected most of our cancers to show choline on both the in vivo and in vitro studies. However, our in vivo MRS data confirmed by in vitro MRS shows that choline in breast cancer is only present in slightly more than half (57%) of breast cancer patients, and suggests that not all cancers contain choline that is measurable by in vivo or in vitro MRS scans. Of note, most (6/8) of our positive in vivo scan data was confirmed by the in vitro studies. Negative in vitro scan data in women with positive in vivo MRS scans might be explained by the composition of the targets sampled by fine-needle aspiration. One of the negative in vitro studies might have been due to an extremely bloody sample during fine needle aspiration, yielding few cancer cells. The other negative in vitro study might have been due to needle sampling of the mucinous portion of this patient's mucinous carcinoma, which contains a gelatinous substance and very few cancer cells, resulting in a negative in vitro MRS scan.

43% (6/14) of our cancer patients had technically satisfactory but negative in vivo MRS scans, and 4 of 5 of these patients were also negative on the in vitro MS studies. One negative in vivo MRS scan occurred in a woman with invasive ductal and lobular histology. Invasive lobular cancer usually infiltrates the breast in single cells, spreading through a large amount of breast tissue and fat in files with little or no discernable mass. We postulate that the negative in vivo MRS scan in our patient with ductal and lobular histology may have occurred because of the infiltrative invasive lobular histology portion of this breast cancer. Baker et al found no choline metabolites, even at 4T, in a case of lobular cancer[25]. Why did this tumor show choline on the in vitro study? The needle may have sampled an extremely cellular portion of the tumor, resulting in the elevated choline peak.

An alternative explanation for the negative in vivo scans in cancer patients would be that the tumors were too small to be detected by the in vivo MRS sequence, since the negative in vivo scans were in tumors of an average size of 2.62 cm (range 1.8 cm- diffuse, the average size calculated on measurable tumors) compared to the positive scans in tumors of an average size of 4.3 cm (range 1.5 – 8 cm). However, this would not explain the high correlation of the negative (83%) in vitro MRS confirmatory studies.

One of the limitations of our study was the use of *in vivo* FNA in our patients to obtain breast cell samples, compared to FNA sampling in excised breast specimens in the study by MacKinnon et al. The breast changes configuration between the prone MRI imaging, the upright mammogram, and the supine position in which ultrasound is performed, changing the apparent location of the breast lesion within it. However, during our study, we took extreme care to ensure that the FNA samples were obtained from the same place that the *in vivo* ^{1}H MRS was obtained, usually by determining the abnormality location and morphology on the scans, and correlating the lesion with the lesion on mammogram/ultrasound/physical examination. These types of correlations and the ultrasound-guided FNA were done by invasive breast imaging specialists, who perform these types of correlations and biopsies in daily routine work, ensuring that the imaging-detected lesion and the biopsied lesion were the same. However, there second, inherent problem with *in vivo* FNA cytology in clinical use is the possibility of inadequate sampling, reported as a sampling error as high as 15% for enough cellular material to make a definitive cytologic diagnosis. This may have played a role in the patients with cancers that classically have fewer cells per volume of tissue, for example, the mucinous, lobular or inflammatory carcinomas, depending on where the needle samples were obtained, and may have led to no choline peak in our *in vitro* samples. Third, even though we used local anesthesia, one cannot sample the tumor in patients as vigorously as in excised breast specimens due to pain and bleeding in patients. This may also explain some of the differences between our study and that of MacKinnon's group.

Our results were as expected in patients with benign disease on the technically satisfactory in vivo

MRS studies, and were negative in 3 of the 4 in vitro studies. In 3 cases there were elevated in vitro choline peaks in fibrocystic change and fibrosis, and in patients with a hamartoma and silicone granulomas. On retrospective review of the fibrocystic change/fibrosis case, we re-confirmed that the lesion location on MRI, the in vivo MRS, the mammogram, the FNA ultrasound images correlated to the location of the fine-needle aspirate and the tissue removed at surgical biopsy. Why did breast cells from surgically proven benign fibrocystic change and fibrosis show an elevated in vitro choline peak? It has been reported that surgically induced hypoxia during the FNA extraction process which may lead to catabolism of Ptdcholine, and since free choline is one of the breakdown products; this could lead to increased choline in vitro[3]. Possibly the trauma of the FNA process may have led to the elevated choline peak in this particular patient. Choline can also be detected in healthy breast-feeding patients. In this population, elevated choline peaks might be expected since increased metabolic activity also results in increased choline [8,26], however our patient was not nursing at the time of her scan. Either of these factors or possible natural physiologic fluctuations in choline levels due to hormonal cycles may have contributed to the in vitro elevated choline peak in our patient. In the hamartoma and the patient with silicone injections it is possible that the choline may have been due to increased cell turnover, perhaps from inflammation in the case of the silicone injections.

In summary, our data show that in vivo MRS detects choline in 57% of breast cancers in which we had confirmation of results by in vitro studies, but there are possible clinical situations in which MRS may not be applicable or may be falsely negative or positive. Although MRS is a promising technique, its applicability as a clinical diagnostic tool to breast MRI is premature. Technical limitations of this technique include patient motion during scanning, and difficulties in lipid suppression, which can result in poor scan results.

Currently, we are working on the transfer of the MRS pulse sequences to a 3T high field system located at the Lucas Center for Magnetic Resonance Spectroscopy and Imaging on the Stanford Campus. The higher field strength should provide improved performance due to both increased SNR and greater spectral peak separation. Spectroscopic data has a higher signal to noise ratio in higher magnetic field strengths, yielding more information on key metabolites. This is especially important in the breast, where fat and water interfere with the choline peak, and use of MRS at higher field strengths may yield more promising results.

KEY RESEARCH ACCOMPLISHMENTS:

1) MRS pulse sequence development, with additional PRESS sequence modification with the addition of dual-band BASING pulses for added water and lipid suppression
2) The addition of up to 6 graphically prescribed highly selective spatial saturation bands resulting in sharper delineation of the excited volume of tissue
3) Determination of optimal RF coil configurations (4-coil phased array breast coil configured to only receive signal from the right or left side)
4) Improved data reconstruction and quantification algorithms using custom software to both detect and correct motion-induced phase variations followed by peak integration.
5) Processing of in vivo MRS and in vitro MRS scans using custom software.
6) Correlation and analysis of resulting MRS data with pathology and cytology findings.
7) Preliminary work on 3T high field strength MRS pulse sequence development

REPORTABLE OUTCOMES:

Personnel receiving pay from this research effort

Debra M. Ikeda, M.D.
Daniel Spielman, PhD
Josh Star-Lack, PhD
Sandeep Hunjan, PhD
Leslie Roche, RN

Peer-reviewed Journals

1. Star-Lack JM, Adalsteinsson E, Gold GE, Ikeda DM, Spielman DM. Motion correction and lipid suppression for 1H magnetic resonance spectroscopy. Magn Reson Med, 2000. 43(3): p. 325-30.3
2. Rausch-Garrity P, Hunjan SS, Spielman D, Adalsteinsson E, Star-Lack J, Sawyer-Glover A, Ikeda DM. Comparison of in vivo and in vitro 1H magnetic resonance spectroscopy and contrast-enhanced breast MRI of breast cancer and benign breast disease. In preparation.

Abstracts

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2. Spielman D, Hunjan S, Sawyer-Glover A, Adalsteinsson E, Ikeda DM. "Proton Spectroscopic Imaging of Breast Cancer". International Society for Magnetic Resonance in Medicine, April 2001, Glasgow, Scotland
3. Rausch-Garrity P, Spielman D, Hunjan S, Sawyer-Glover A, Adalsteinsson E, Ikeda DM. "Dynamic Spiral Imaging K21 Values and Breast Cancer Morphology on High-Resolution 3DSSMT MRI Scans: Correlation with Proton Spectroscopic Imaging of Breast Cancer". International Society for Magnetic Resonance in Medicine, April 2002, Honolulu, Hawaii.
4. Ikeda DM Spielman D, Hunjan S, Sawyer-Glover A, Adalsteinsson E. "Magnetic resonance spectroscopy of breast cancer" . 2002 Era of Hope Meeting. Orlando, Florida. September 25-28, 2002.

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CONCLUSIONS:

We have developed a unique magnetic resonance imaging multi-voxel pulse sequence producing spectroscopic images of key metabolites found in breast cancer, and validated our work with in vitro spectra and pathology. We have shown that choline peaks are present in 57% of breast cancers, and that our MRS sequence was unaffected by intravenous contrast, increasing its clinical utility as an adjunctive study to clinical breast MRI scans. However, MRS may have a major limitation in that specific tumor histologies that have dispersed cells, such as carcinomas with invasive lobular carcinoma features, may not show choline peaks. Furthermore, contrary to other investigators' research, our study shows that many invasive ductal cancers do not contain choline as a detectable metabolite. Also, benign breast conditions, including fibrocystic change/fibrosis, hamartomas and patients with silicone injections may have detectable choline from increased metabolic activity but not due to malignancy. Thus, technical developments to date have significantly contributed towards the goal of making MR spectroscopic imaging a clinically useful procedure that could be implemented at the time of a contrast-enhanced MRI scan, but would be helpful only in choline positive cases to prompt biopsy. Important applications for this technique include distinction of a breast cancer recurrence from a post-biopsy scar within 24 months of initial breast cancer treatment, or evaluation of unexpected enhancing lesions on contrast-enhanced scans obtained for a specific target lesion.

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Appendix 1

Images from Magnetic Resonance Imaging studies of a woman with a large untreated breast cancer, and corresponding in vivo and in vitro spectroscopy data showing choline peaks expected in breast cancer, and not present in normal breast tissue.

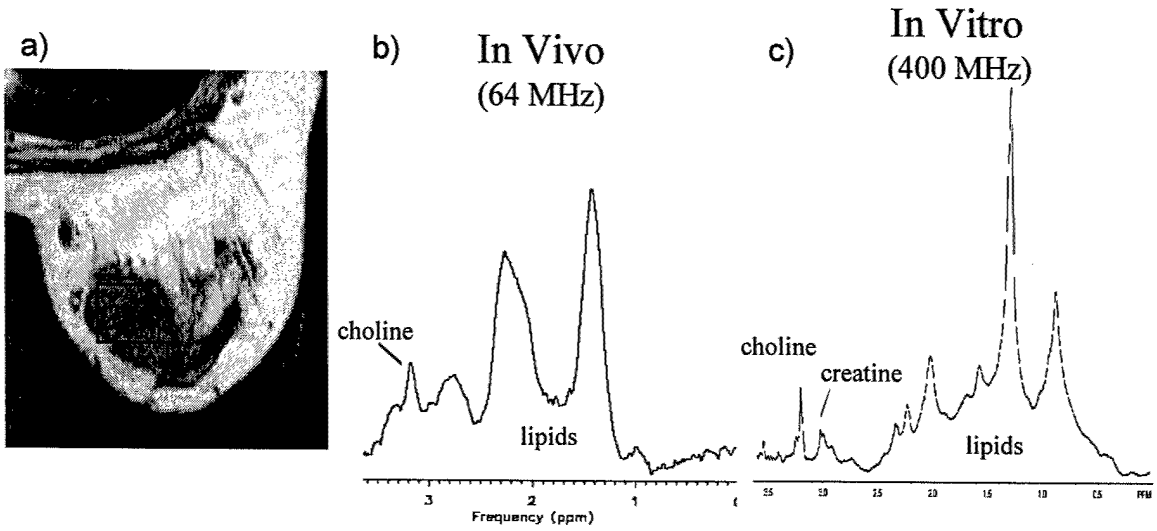

Figure 1. Single voxel MRS of breast cancer of a woman with a large invasive ductal cancer. (a) T1-weighted image showing location of 3x3x3 cm^3 spectroscopic voxel. (b) spectrum corresponding to voxel shown in (a) collected with the following parameters: PRESS localization, TR/TE = 2000/144 ms, 64 averages, water and lipid suppression via BASING technique. (c) 400 MHz in vitro spectrum from fine needle aspirate of the lesion shown in (a) (TR=4s, 1024 FIDs). Note, no lipid suppression is used in (c) in contrast to the in vivo data (b) in which saturation pulses partially suppress the 1.3 and 0.9 ppm lipid peaks. Choline (3.2 ppm) is visible in both in vivo and in vitro spectra.

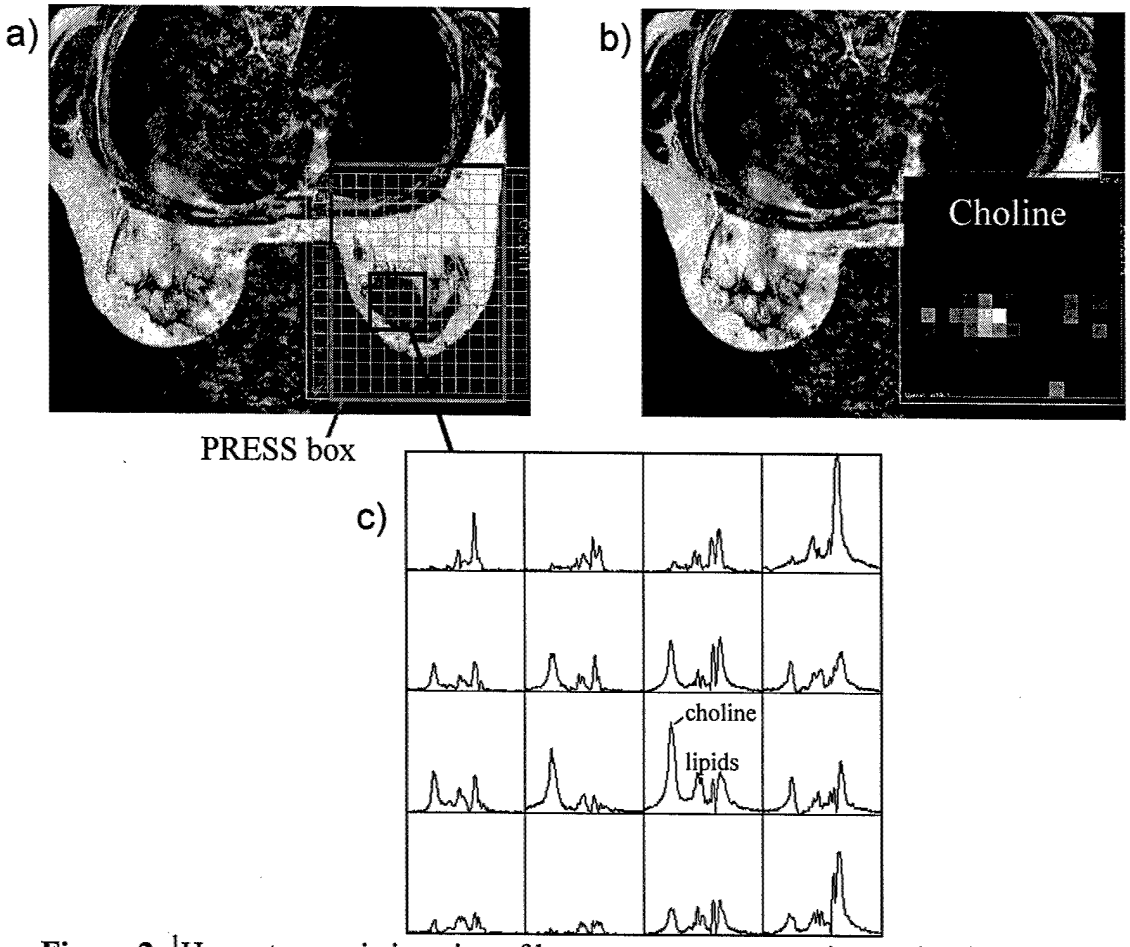

Figure 2. ^{1}H spectroscopic imaging of breast cancer, same patient as in Figure 1. (a) T1-weighted imaging showing PRESS box and corresponding 16x16 array of 1cc voxels. (b) metabolic map of the choline peak overlayed on the T1-weighted image. (c) spectra from subset of voxels highlighted in (a). High choline is visible within the lesion.

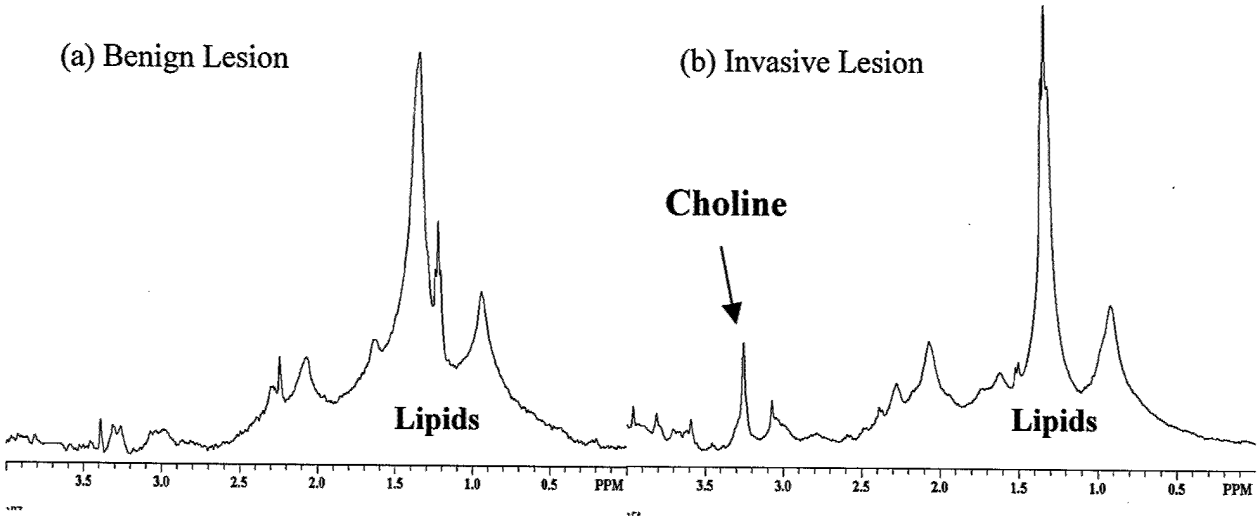

Figure 3. *In vitro* ^{1}H MR spectra (256 acquisitions) acquired at 400 MHz of FNA samples from a benign (a) and a malignant (b) breast mass. Both spectra are dominated by signals originating from lipids: methyl (-CH3); methylene (-CH2-) from the fatty acids of triglycerides and phospholipids; α- and β-methylene (2.3 ppm); and oleffenic (-CH=CH-) resonances occurring at 0.9, 1.3, 2.3 5.3 ppm, respectively. Figure 3b shows a spectrum from a cancer patient, which exhibited an increase in the N-trimethyl resonance of choline at 3.2 ppm, arising predominantly from the choline of phospholipds (creatine at 3.05 ppm).

Appendix 2

Table 1. In vivo and in vitro MRS detection of choline compared to pathology in 21 patients with 22 breast lesions*

(a) Pre-Treatment patients

Patient	Pathology	in vivo	in vitro	Size at MR	Therapy Status
1	IDC with mucinous features	+	_	1.5 cm	None
2	IDC Grade II	_	_	2.3 cm	None
3	IDC/inflammatory	+	+	8 cm	None
4	IDC	N/A	+	diffuse	None
5	IDC grade III/inflammation	-	-	3 cm	None
6	IDC	+	-**	4.5	None
7	IDC	N/A	+	1.5	None
8	IDC	+	+	2.9	None
9	IDC	-	-	3.5	None
10	IDC	-	-	1.8	None
11	IDC	-	-	2.5	None

(b) Post-Treatment Patients

Patient	Pathology	in vivo	in vitro	Size at MR	Therapy Status
12	IDC and ILC	_	+	DIFFUSE	Chemo
13	IDC	+	+	6 cm	Chemo
1	IDC	+	+	6 cm	Chemo
15	IDC	+	+	3.3	Chemo
16	IDC	+	+	2.8	Chemo/XRT

(c) Patients with Benign Disease

Patient	Pathology	in vivo	in vitro	Size at MR	Therapy Status
17	Scar Tissue	–	–	2 cm	Chemo+Rad
18	Fibro Adenoma	–	–	1.5 cm	None
19	Fibrocystic Change and Stromal fibrosis	–	+	2 cm	None
20	Phyllodes tumor	-	-	2 cm	None
21	Hamartoma (neg FNA)	N/A	+	3 cm	None
22	Silicone granuloma (neg FNA)	N/A	+	diffuse	none

IDC= invasive ductal cancer, ILC=invasive lobular cancer

*4 patients had technical problems including motion and poor lipid suppression. Lesions 8 and 20 are in the right and left breast of the same patient

** Very bloody sample

In vitro and in vivo results are indicated as a positive or negative for an increase in the N-trimethyl resonance of choline at 3.2 ppm (arising predominantly from the choline of phospholipids).